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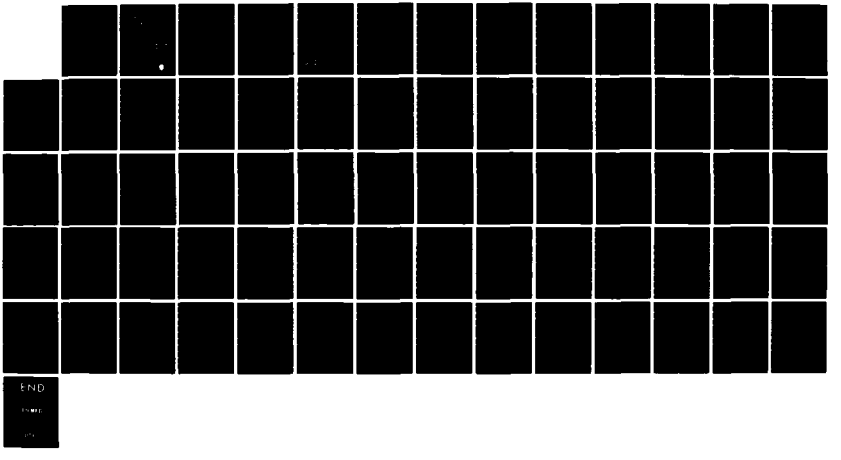

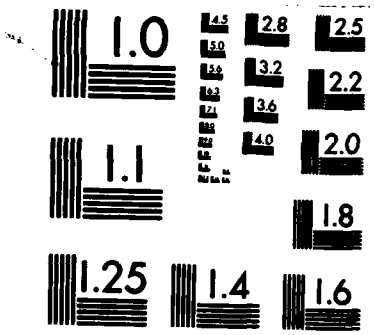

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EFFECTS OF LONG-TERM LOW-LEVEL RADIOFREQUENCY RADIATION EXPOSURE ON RATS

VOLUME 8. EVALUATION OF LONGEVITY, CAUSE OF DEATH, AND HISTOPATHOLOGICAL FINDINGS

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For 25 months 100 male SPF rats were exposed to pulsed 2450-MHz circularly polarized microwaves, at an average power density of 0.48 mW/cm^2; another 100 rats served as sham-exposed controls. Evaluation of survival time showed no statistically significant difference during any phase of the life span. There was no association between a specific cause of death and the treatment condition. Except for rats that died of urinary tract blockage, there is some indication that the survival times were longer in the exposed animals. Of 1992 nonneoplastic lesions, only glomerulonephropathy was less frequently observed in the exposed animals; no other lesions differed statistically. Of 192 neoplastic lesions observed, no specific increase was seen in any specific organ or tissue. Collapsing of the data and an analysis with respect to the occurrence of all neoplasms showed no difference for benign lesions, but a statistically higher incidence of primary malignancies in the exposed animals than in the sham exposed. The biological significance of this finding is questionable at this time.

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INTRODUCTION

The increased use of microwave-emitting electronic devices for various purposes in consumer, military, medical, and industrial areas has resulted in the long-term low-level exposure of a significant proportion of the human population. More than 6,000 articles on the biological effects of microwave radiation have been published; however, whether this exposure represents a human health hazard remains unclear (Czerski et al., 1974; Glaser and Dodge, 1975; Tyler, 1975; Justesen and Guy, 1977; Justesen and Baird, 1979; Gandhi, 1980). In most research projects to date, exposure durations have been relatively short and few animals have been exposed; thus little insight has been gained into questions about potential long-term cumulative biological effects.

During the past three years, the Bioelectromagnetics Research Laboratory at the University of Washington has conducted the largest single study ever made of the long-term effect of microwave exposure. The goal of the project was to investigate purported adverse health effects from long-term exposure to pulsed-microwave radiation. The approach was to expose a large population of experimental animals to microwave radiation throughout their lifetimes and to assess any cumulative effects on general health and longevity.

This technical report, the eighth in a series reporting the conduct and results of the study, contains the histopathological findings, cause of death, and effect on longevity. This is the first comprehensive histopathological study of the bioeffects of microwaves on animals after long-term exposure to pulsed-microwave radiation. This project was designed to record neoplastic and nonneoplastic lesions and to detect any differences between the control and exposed animals with respect to frequency of occurrence of these lesions and age at death of the animals.

Sprague-Dawley rats were used to derive an inference regarding the hazards of long-term exposure to RFR for human populations. The protocol for this experiment required extensive histopathological examination to reveal all possible morphological lesions and to help provide a definite diagnosis for any disease condition. It is important to evaluate spontaneous lesions in aging rats, to document these lesions, and to use this data to help explain abnormal biochemical, behavioral, metabolic, and immunological test results. The histopathological data is part of a multiple-panel profile that has proved useful to researchers for diagnosing and understanding abnormalities in experimental animals. A profile of multiple biological parameters aids in evaluating the animals for unsuspected organ-system malfunction and can help define the problem in animals with subclinical or undiagnosed abnormalities. The profile permits a more complete understanding of the pathophysiology of abnormal or diseased states and demonstrates multisystemic organ involvement that is often missed when only individual tests or isolated biological parameters are selected and measured.

METHODS

Experimental Animals

Failure to define and control environmental and disease variables can complicate or invalidate experimental results, particularly where long-term studies are performed (Anver and Cohen, 1979; Cohen, 1979); thus this project required animals free of infectious diseases. Knowledge of the spontaneous lesions that can develop in a chosen strain is also essential to interpretation of the experimental results (Finch, 1977; Hollander, 1973; Levine and Deshpande, 1980). To standardize the environment of the experimental animals and control their general health status, we acquired a colony of cesarean-derived barrier-reared (BR) animals from Camm Research Institute, Inc., 414 Black Oak Ridge Road, Wayne, NJ 07470. The rats were serologically tested at Yale University, indicating that the colony was free of specific pathogens. The rats had the following defined microflora:

Lactobacillus casei ssp. *rhamnosus*
Lactobacillus acidophilus
Bacteriodes fragilis ssp. *ovatus*
Streptococcus faecalis ssp. *liquefaciens*
Streptococcus lactis ssp. *diacetilactis*

We maintained the animals under specific-pathogen-free (SPF) conditions throughout the study. For a detailed description of the project's animal maintenance procedures, see Volume 1 of this series.

The Sprague-Dawley (SD) rat has a rapid growth rate and reaches an average weight of 360 g (male) by the 84th day. This large size was important because of the frequency and volume of the blood samples drawn. The gentle disposition of the animal was also important. The SD rat has been used in scientific research for more than 50 years. This outbred strain was developed by R. W. Dawley and for many years was produced commercially by Sprague-Dawley, Inc., Madison, Wisconsin (Foster, 1980; Lindsey, 1979). These animals have a heterogeneous genetic background, as does the human population.

Four hundred 21-day-old weaned male rats were obtained from the Camm Research Institute and placed in an SPF quarantine facility. During this period each animal was physically examined and toe-clipped for individual identification. Ten animals were randomly selected from the colony for a general health screen; 50 other animals were randomly assigned to be used for initial establishment of the immunology test procedures; and 20 others were removed to provide baseline values for a whole-body carcass analysis. Seven days after arrival, 250 animals were bled to obtain individual serum chemistry and hematology baseline values. The next day 200 of the animals most uniform in size were randomly assigned to the two treatment groups (100, exposed; 100, sham exposed) and began waveguide adaptation, in which they experienced the same daily procedures used throughout the study but without actual microwave exposure. Microwave exposure of the exposed group began when the rats were 7 weeks old, after 3 weeks of exposure chamber adaptation.

The rats were housed in custom-designed individual polypropylene cages, each within a circular waveguide. These individual cages had plastic rod floors and did not require bedding. The rats were removed from these cages daily during cleaning and were placed in individual filter-bonneted polycarbonate cages located in the same room. These cages contained bedding of autoclaved ground-up corn cobs that was changed 1-2 times per week. The bedding used in the holding cages was assayed by Raltech Scientific Services (P.O. Box 7545, Madison, WI 53707) for arsenic; cadmium; lead; mercury; aflatoxins B_1, B_2, G_1, and G_2; and 10 pesticides. Within the SPF room, the cages and exposure chambers were within a laminar-flow alcove where the airflow rate was approximately 22 exchanges each hour. The cages had an ambient temperature of 21 $\pm$ 1°C, which was uniform throughout the facility, and a humidity of 45-60%. There was a 12/12-h light/dark cycle in the animal rooms. Sterile water was supplied in custom bottles, and the rats were fed rat chow ad libitum (Purina Certified Autoclavable Rodent Chow #5014: crude protein, min. 20%; crude fat, min. 4.5%; crude fiber, max. 5.5%; and ash, max. 7.0%). This chow was assayed after autoclaving by Ralston Purina Company (Checkerboard Square, St. Louis, MO 63188) and double checked by Raltech Scientific Services for acceptable levels of required nutrients.

Facility

As part of this project, a unique exposure facility was constructed that allowed 200 rats to be maintained under SPF conditions while housed in individual circularly polarized waveguides. This facility has been described in detail in Volume 1 of this series; we will only briefly discuss it here. The exposure facility consisted of two rooms, each containing 50 active-exposure waveguides plus 50 sham-exposure waveguides for control subjects. Each room contained two 2450-MHz pulsed-microwave generators, each capable of delivering a maximum of 10 W average power at 800 pps with a 10-μs pulse width. This carrier was square-wave modulated at an 8-Hz rate. The power distribution system delivered 0.144 W to each exposure waveguide, for an average power density of 0.48 mW/cm^2. Whole-body calorimetry, thermographic analysis, and power-meter data analysis indicated that these exposure conditions resulted in average specific absorption rates (SARs) ranging from approximately 0.4 W/kg for a 200-g rat to 0.15 W/kg for an 800-g rat.

Throughout the study all surviving animals were sampled for blood at regular intervals; and serum chemistries, hematological values, protein electrophoretic patterns, thyroxin (T_4), and plasma corticosterone levels were determined. Body weight and food and water consumption were measured daily, and oxygen consumption and carbon dioxide production were measured periodically on a subpopulation of exposed and sham-exposed animals. Activity was assessed at regular intervals in an open-field apparatus throughout the study. After 13 months 10 rats from both treatment conditions were killed for immunological competence testing, whole-body analysis, and gross and histopathological examinations.

Pathology Procedures

Gross and histopathological examination of 10 rats, 21 days old, was performed at the time of animal procurement as part of a general health screen. After 13 months 10 exposed rats and 10 sham-exposed controls were randomly selected and killed for examination. At 25 months the surviving 12 exposed rats and 11 sham-exposed controls were killed and examined. In

addition to these 43 animals, 157 animals were examined that died spontaneously or were terminated in extremis during the study.

Animals dying spontaneously during the study, aside from the two scheduled kills, were immediately refrigerated and necropsied with the tissues fixed as soon as possible to minimize autolysis. A night check between 2200 and 2300 was made to detect spontaneous deaths, thus minimizing postmortem autolysis. After showering and dressing in sterile apparel, the technician entered the dark colony rooms and, using a nonarousing red light, examined each animal. The rats were quite active at this time and their movements could be easily detected. If a dead animal were found, the technician performed a partial necropsy consisting of a longitudinal incision along the ventral midline from the mouth to the tail. All abdominal and thoracic organs were exposed; the brain and kidneys were removed and sectioned; slices were made in the heart, liver, and testicles to insure proper fixation; and the lungs were infused with formalin via the trachea. The carcass was then submerged in a 4000-cm^3 container of 10% buffered formalin. A complete gross examination and collection of specimens for histological examination was performed the next morning.

For the 13th- and 25th-month kill, the rats were removed from the exposure chambers between 0730 and 0800 and placed in a holding area adjacent to the necropsy room. Starting at 0800 the animals were brought one at a time, at 1-min intervals, into the necropsy room where they were anesthetized in a Halothane-nitrous oxide-oxygen chamber and killed by rapid exsanguination via the carotid and brachial arteries. This method minimized anoxic or agonal hemorrhages and hypostatic congestion. The spleens were immediately removed aseptically; 5-8% of the spleen was saved for histopathological examination and the remainder was placed in a sterile dish on ice for immunological studies. Complete necropsies were performed on all 200 experimental animals (Appendix A, Necropsy Technique for Male Rats). Representative sections from the following tissues were fixed in 10% buffered formalin, paraffin processed, sectioned at 5 μm, and stained with hematoxylin and eosin for light microscopy:

adrenals
brain
bone marrow
bronchi
cecum
colon
duodenum
epididymus
esophagus
eyes
harderian glands
heart
ileum
jejunum
kidney
liver
lungs
lymph nodes
  cervical
  mesenteric
middle ears
nasal passages
pancreas
parathyroids
pituitary gland
prostate
right stifle joint
salivary glands
  parotid
  sublingual
  submaxillary
seminal vesicles
skeletal muscle
skin
spleen
stomach
testicles
thymus
thyroid
trachea
urinary bladder
grossly evident lesions

Also, organ weights were obtained for the kidneys, adrenals, testicles, heart, and brain. These data are reported and discussed in Volume 7--Metabolism, Growth, and Development.

All tissues were placed in "Tissue-Tek II" cassettes, with appropriate log numbers legibly printed on the diagonal surface for processing. Each cassette contained only the tissues that were to be embedded together for microtoming. In general, tissues were embedded so as to present the largest possible surface area for microtoming, and thus for microscopic examination. Exceptions to this were tubular organs, such as trachea and intestine, which were embedded so as to present a cross-section of the entire circular dimension of the organ at microtoming. When multiple samples of an unusual finding were taken for processing, it was advantageous to embed several samples to present several different surfaces for microtoming, thus allowing the pathologist to examine the lesion microscopically from different views.

The cassettes were placed in a basket and cycled through an Auto-Technicon Ultra Tissue Processor. The processing cycle includes 30 min in each of the following: 70% ethyl alcohol, 80% ethyl alcohol, 95% ethyl alcohol, 95% ethyl alcohol; 100% ethyl alcohol, 100% ethyl alcohol, 100% ethyl alcohol, clearing reagent, clearing reagent, and paraffin. This was followed by 1.5 h in a vacuum paraffin bath. The tissues were then embedded in steel molds using a Tissue Tec II embedding center.

All routine sections were microtomed at 4-6 μm on an AO Spencer "820" microtome and stained with hematoxylin and eosin. Available stains included oil-red-O, Gomori's trichrome, Gomori's methenamine silver, Brown and Brenn, Giemsa, Congo red, Prussian blue, Weigert's fibrin, Wilder's reticulin, Gomori's aldehyde fuchsins, PAS, Ziehl-Neelson acid fast, Luxol fast blue, and other special stains as needed for characterization and interpretation of lesions (Luna, 1968).

Parasitology

The 10 rats killed for the initial health screen and 40 of the 43 rats from the two interim kills were examined for parasites. An anal tape impression from each animal was examined microscopically for pinworm ova. Fecal pellets from each were suspended in a saturated sodium nitrate solution, and a coverslip flotation preparation was examined for endoparasite ova and cysts. A 2- x 3-cm piece of skin from the back and neck region was removed at necropsy, placed in a petri dish, and examined under a dissecting microscope for ectoparasites. Sections of skin and the gastrointestinal tract were examined histologically from all of the experimental animals.

Microbiology

A section of the trachea and the right cranial lobe of the lung were removed aseptically from the initial 10 health-screened animals and the 20 animals from the first (interim) kill. The aseptically collected specimens were placed in transport media (trypticase soy broth, 0.5% BSA, and 200 units penicillin) and were frozen and shipped to Microbiological Associates (Bethesda, MD) for mycoplasma culture. The 20 animals from the final kill were screened for mycoplasma infection serologically, using the ELISA Test, by the Department of Animal Medicine at the University of Washington. The department used standard bacteriologic techniques to culture a calcium alginate swab of terminal colon contents and a section of the left diaphragmatic lobe of the lung.

Serology

Serum samples from the 10 health-screened and the 20 interim-kill rats were heated for 30 min at 56°C, diluted 1:5 with physiological saline, and shipped to a commercial laboratory (Microbiological Associates) for detection of antibodies to the following rodent viruses: hemoglutination inhibition antibodies to pneumonia virus of mice (PVM), reovirus-3, GDV II (mouse poliovirus), sendai virus, Kilham rat virus (KRV), and Toolan's H-1; and detection of complement fixation antibodies to mouse adenovirus, mouse hepatitis virus (MHV), lymphocytic choriomeningitis virus (LCM), and rat coronavirus (RCV). A serological test for these viruses and enzyme-linked immunosorbent assay (ELISA) for Mycoplasma pulmonis were performed on 20 final-kill animals by the Department of Animal Medicine at the University of Washington.

Statistical Analysis of Data

The pathology consultant provided animal-evaluation data to the Bioelectromagnetics Research Laboratory, which was responsible for computer entry and quality control. The statisticians then evaluated the data, and the final results were reviewed by the pathologist for appropriate interpretative comments.

The occurrence of nonneoplastic and neoplastic lesions was recorded along with the age of the animal and whether the animals had died spontaneously or were sacrificed. The cause of death was also recorded for each animal. The pathological data were collected to compare exposed and sham-exposed groups' survival curves, age-associated lesions, incidence of tumor metastasis, and the occurrence of multiple lesions per rat.

Cumulative survival curves for the exposed and sham-exposed animals were estimated using product-limit estimates (Kaplan and Meier, 1958) and compared using the log-rank statistic (Mantel, 1966). The histopathology data were grouped with respect to the age, at 6-month intervals, and the data were divided into neoplastic and nonneoplastic diagnoses. The incidence of neoplastic or nonneoplastic lesions is given as the proportion of the number of animals bearing such lesions at a specific anatomic site

(numerator) to the number of animals examined pathologically (denominator). For tissues that required gross observation for detection of lesions (i.e., skin or subcutaneous tumors), for lesions that appeared at several sites (i.e., multiple lymphomas), or for tissues that were examined histologically only when lesions were detected grossly, the denominator consisted of the number of animals necropsied in that experimental group.

The analysis of the lesions involved a 4-way table with factors of age at death, treatment condition, mode of death (terminated or spontaneous), and organ. The tables were then collapsed with respect to individual organs. From these tables the Mantel-Haenszel estimate of the odds ratio was computed, and the Chi-square statistic was used to test whether or not the odds ratio was significantly different from 1 (Mantel and Haenszel, 1959). This statistic reflects the difference in prevalence of lesions, over time, between the exposed and sham-exposed animals. If an animal had malignant lesions, its time-to-death was taken as its true survival time. If there were no malignant lesions present, the time-to-death was considered censored. The log-rank statistic was used to compare these "survival times" of the exposed animals with those of the sham-exposed animals (McKnight and Crowley, 1984).

RESULTS

Life Expectancy

An analysis of mortality was completed prior to analysis of the histopathological data. Survival curves for exposed and sham-exposed animals are presented in Fig. 1. The survival curves were compared using the log-rank statistic (Chi-square = 0.355, p = .5513, df = 1), yielding the conclusion that no significant effect existed.

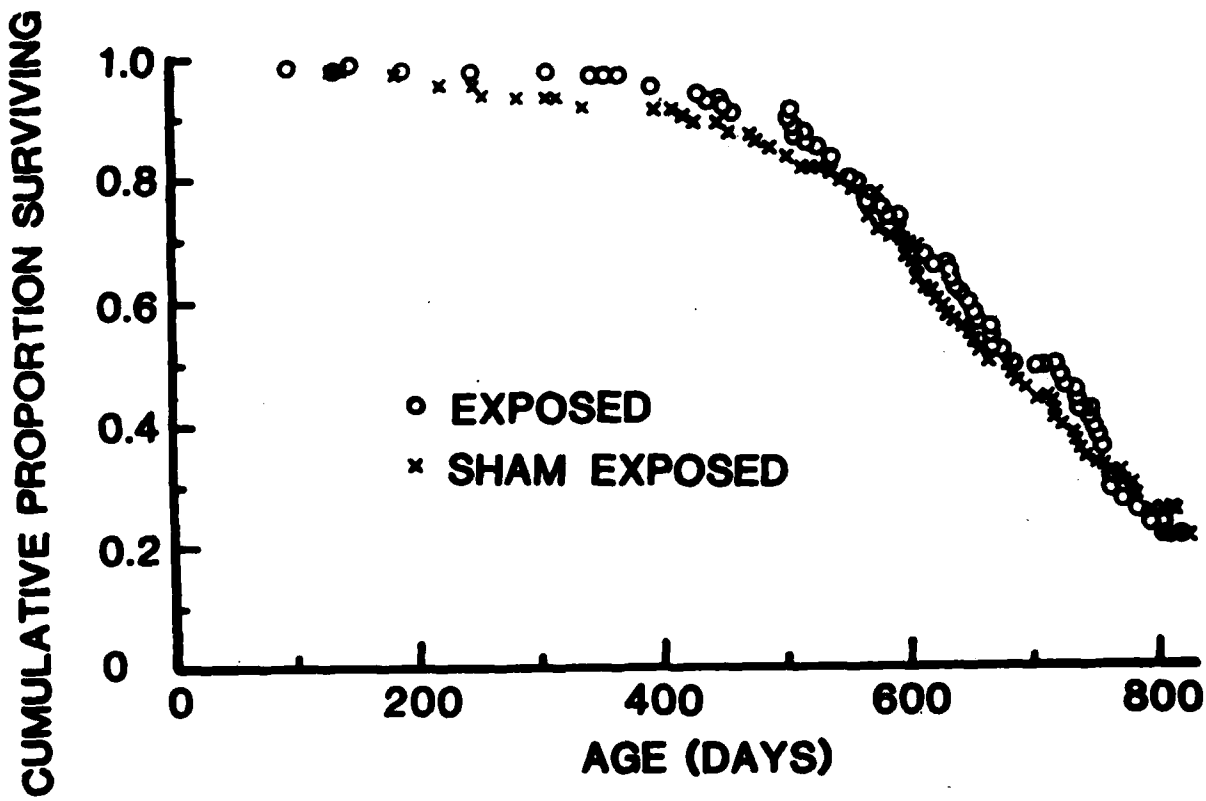

Figure 1. Cumulative survival for exposed and sham-exposed animals throughout the 25-month study.

Age at Death

To simplify the analysis of the histopathology data with respect to the age of the animal, age at death was categorized in 6-month intervals (e.g., 1-6, 7-12, 13-18, 19-24, and 25-30 mo). Mode of death was categorized as being either spontaneous (due to natural disease processes) or terminated (due to experimental procedures such as anesthetic overdose or euthanasia). A mortality summary--age, treatment condition, and mode of death--is presented in Table 1. The mortality frequencies represented in these 20 combinations were used throughout the following analyses of the histopathological lesion frequency.

TABLE 1. SUMMARY OF MORTALITY FREQUENCY AS A FUNCTION OF AGE, TREATMENT CONDITION, AND MODE OF DEATH

		Age (months) at death					
		1-6	7-12	13-18	19-24	25-30	Total
Exposed:	Terminated	2	3	13	6	16	40
	Spontaneous	1	2	12	29	16	60
Sham:	Terminated	1	3	12	1	18	35
	Spontaneous	2	5	11	34	13	65
Totals		6	13	48	70	63	200

Cause of Death

A summary of the primary causes of death for each treatment group is presented in Table 2.

TABLE 2. SUMMARY OF PRIMARY CAUSES OF DEATH FOR EXPOSED AND SHAM-EXPOSED ANIMALS

Cause of Death	Exposed	Sham
Glomerulonephropathy	17	15
Urinary tract blockage	9	19
Atrial thrombosis	7	9
Pituitary adenoma	4	8
Bleeding death	5	9
Cardiomyopathy	4	2
Asphyxiation	3	0
Generalized lymphosarcoma	3	1
Degenerative vac. encephalitis	3	2
Pituitary carcinoma	2	0
Nephroblastoma	1	1
Enteroliathisis	1	0
Adrenal carcinoma	1	1
Pancreatic adenoma	0	1
Hemangiosarcoma	1	0
Abdominal liposarcoma	1	0
Gastric squamous cell papilloma	1	1
Cardiac neurinoma	0	1
Congestive heart failure	1	1
Pyelonephritis	1	0
Cerebral thrombosis	1	0
Trans. cell carcinoma	1	0
Hemorrhage, cystitic	0	1
Gastric hyperkeratosis	1	0
Cerebral hemorrhage	1	0
Myocardial hypertrophy	0	1
Thymic lymphosarcoma	1	1
Aud. seb. carcinoma	1	0
Hemopericardium	1	0
Squamous cell carcinoma	1	0
Chronic suppurative nephritis	0	1
Interim kill	10	10
Final kill	12	11
Unknown	5	4
	100	100

For most of the categories the data are sparse, so a Chi-square analysis would be inappropriate. The following list identifies the major causes of death (at least five deaths for each group--sham or exposed):

	Exposed	Sham
Glomerulonephritis	17	15
Urinary tract blockage	9	19
Atrial thrombosis	7	9
Pituitary adenoma	4	8
Kills	22	21
Other	41	28
Total Animals	100	100

The Chi-square statistic for association was 7.86 ($p = .17$, $df = 5$), hence the data in the above list do not contradict the hypothesis of no association between cause of death and condition. Although this statistic indicates that there is no association between cause of death and condition, it is still possible for death to have occurred earlier in one group than in the other.

To check this we completed a survival-type analysis for glomerulonephritis, atrial thrombosis, urinary tract blockage, and pituitary adenoma. Based on the log-rank statistic, the survival times are the same for both groups for atrial thrombosis, glomerulonephritis, and pituitary adenoma; however for urinary tract blockage, the exposed animals had the longer survival times.

Pathology

During histopathological examination of all the animals, 2,184 observations of pathological change were noted. A complete breakdown of these observations is presented in Appendix B indicating the treatment group, age, mode of death, and total nonneoplastic and neoplastic observations for each animal. Analysis of the data was divided into two parts: nonneoplastic and neoplastic diagnoses. A lesion glossary is listed in Appendix C.

Nonneoplastic Lesions

A total of 1,992 nonneoplastic pathological observations were made, covering 217 unique combinations of organ and lesion identifiers. Appendix D presents a summary of this information, indicating the total number of specific diagnoses for each organ and lesion combination. A further reduction of the data (Table 3) indicates the number of observed lesions for each organ system.

TABLE 3. SUMMARY OF TOTAL NONNEOPLASTIC DIAGNOSES BY ORGAN SYSTEM AND TREATMENT CONDITION

Organ	Exp.	Sham	Organ	Exp.	Sham
Acc. genital	6	3	Middle ear	1	2
Adrenal	85	79	Nasal cavity	40	21
Blood vessel	43	32	Pancreas	3	3
Brain	14	7	Parathyroid	2	2
Cecum	3	1	Parotid SG	8	3
Cerv. l. node	14	18	Pineal	1	1
Colon	13	12	Pituitary	29	23
Duodenum	1	2	Preputial gland	45	37
Ear	1	0	Prostate	18	22
Epididymus	1	1	Skeletal muscle	2	0
Esophagus	1	0	Skin	5	1
Eye	19	11	Spinal cord	0	1
Harderian gland	2	5	Spleen	45	54
Heart	77	80	Stomach	4	1
Ileum	0	1	Submax SG	0	4
Intestine	3	0	SubQ tissue	0	1
Jejunum	0	1	Testes	23	25
Kidney	97	103	Thymus	2	2
Lacrimal gland	10	3	Thyroid	91	95
Liver	60	37	Trachea	2	0
Lung	182	181	Urethra	3	9
Lymph node	13	11	Urin/Bladder	25	25
Mammary	0	2	Zymbol's gland	39	36
Mesentery	1	0			
Total observations:	Exposed		1034		
	Sham exposed		958		
			1992		

The major nonneoplastic lesions in this study agree with those reported for the aging SD rat by Berg (1967) and Anver, Cohen, Lattuada, and Foster (1982). Ten of the most prevalent nonneoplastic lesions were singled out for detailed analysis to detect any significant differences in the incidence of lesions at age of death between the exposed and sham-exposed groups. These nonneoplastic lesions are glomerulonephropathy, periarteritis, cardiomyopathy, atrial thrombosis, adrenal cellular alteration, arteriosclerosis, testicular atrophy, thyroid atrophy, pituitary cyst, and preputial adenitis.

Chronic glomerulonephropathy is a significant cause of morbidity and mortality in many rat strains. This syndrome has its highest incidence in rats fed an ad libitum commercial diet. The disease has been reviewed by Gray (1977); and although this chronic renal disease can cause clinical illness and death in older rats, its pathogenesis starts at an early age with the initial lesions in the glomeruli. In severe cases--with numerous sclerotic glomeruli, dilated tubules with proteinaceous casts, and interstitial fibrosis--any or all of the following sequelae can occur: elevated blood urea nitrogen (BUN) and creatinine, polydypsia, lowered serum albumin/globulin ratio, hypercholesterolemia, hypertension, fibrous osteodystrophy, hydrothorax, and ascites (Anver and Cohen, 1979).

Table 4 presents a detailed breakdown of the incidence of glomerulonephropathy according to treatment condition, age of the animal at death, and whether death was spontaneous or the result of experimental procedures. Analysis of this data based on a 2 x 2 table of total observations per condition and mode of death yields a Mantel-Haenszel estimate of the odds ratio of .24 with a Chi-square statistic of 4.27 ($p = .04$, $df = 1$). This analysis indicated that glomerulonephropathy was observed significantly less in the exposed animals.

TABLE 4. GLOMERULONEPHROPATHY INCIDENCE/NUMBER OF ANIMALS

		Age (months) at death					
		1-6	7-12	13-18	19-24	25-30	Total
Exposed:	Terminated	1/2	3/3	10/13	6/6	15/16	35/40
	Spontaneous	0/1	2/2	9/12	25/29	16/16	52/60
		1/3	5/5	19/25	31/35	31/32	87/100
Sham Exposed:	Terminated	0/1	3/3	12/12	1/1	18/18	34/35
	Spontaneous	1/2	5/5	10/11	34/34	12/13	62/65
		2/3	7/8	22/23	35/35	30/31	96/100

Periarteritis is an inflammatory lesion of unknown etiology that involves muscular arteries, especially the pancreatic, mesenteric, and testicle vessels. It is characterized by subendothelial edema, damage to the elastic membrane, fibrinoid or hyaline necrosis of the media, and inflammatory cell infiltration into all layers of the arterial wall. These inflammatory cells are most abundant in the adventitia and are composed of numerous neutrophils, lymphocytes, and plasma cells with a few macrophages and eosinophils (Skold, 1961). The onset of this condition was age related; it was diagnosed only three times in the group less than 18 months old, but the incidence greatly increased in animals more than 24 months old. This finding agrees with the findings of Anver, Cohen, Lattuada, and Foster (1982).

Table 5 shows the incidence of periarteritis, broken down by treatment condition, age, and mode of death. The 2 x 2 analysis of incidence by treatment and mode of death yielded a Mantel-Haenszel estimate of the odds ratio of 1.51 with a Chi-square statistic of .57 ($p = .45$, $df = 1$), indicating no differences in the relative occurrence of this diagnosis.

TABLE 5. PERIARTERITIS INCIDENCE/NUMBER OF ANIMALS

		Age (months) at death					
		1-6	7-12	13-18	19-24	25-30	Total
Exposed:	Terminated	0/2	0/3	0/13	0/6	6/16	6/40
	Spontaneous	0/1	0/2	2/12	3/29	6/16	11/60
		0/3	0/5	2/25	3/35	12/32	17/100
Sham Exposed:	Terminated	0/1	0/3	0/12	0/1	6/18	6/35
	Spontaneous	0/2	0/5	1/11	3/34	2/13	6/65
		0/3	0/8	1/23	3/35	8/31	12/100

Chronic cardiomyopathy lesions similar to those described by Fairweather (1967) were present in 28% of the animals. The degenerative myocardial lesions included some or all of the following: myofibril degeneration, atrophy, fibrosis, and mononuclear infiltration. Significant lesions of this type were not prominent prior to 13 months of age. Clinical signs and lesions related to cardiac insufficiency were present in 13% of the animals, all of which were over 5 months old.

The incidence of cardiomyopathy is presented in Table 6, broken down by treatment condition, age, and mode of death. The 2 x 2 analysis of incidence by treatment and mode of death yielded a Mantel-Haenszel estimate of the odds ratio of 1.01 with a Chi-square statistic of .02 ($p = .88$, $df = 1$). This estimate indicates that no difference in incidence existed between the exposed and sham-exposed animals.

TABLE 6. CARDIOMYOPATHY INCIDENCE/NUMBER OF ANIMALS

		Age (months) at death					
		1-6	7-12	13-18	19-24	25-30	Total
Exposed:	Terminated	1/2	1/3	3/13	2/6	5/16	12/40
	Spontaneous	0/1	2/2	2/12	10/29	2/16	16/60
		1/3	3/5	5/25	12/35	7/32	28/100
Sham Exposed:	Terminated	0/1	0/3	4/12	0/1	6/18	10/35
	Spontaneous	1/2	1/5	5/11	10/34	1/13	18/65
		1/3	1/8	9/23	10/35	7/31	28/100

Organizing thrombi were found in the atria of 10% of the animals; the incidence increased with age, becoming significant in animals over 18 months old. The thrombi were usually associated with mild myocarditis of the atrium, or chronic cardiomyopathy.

The incidence of atrial thrombosis is presented in Table 7, broken down by treatment condition, age, and mode of death. The 2 x 2 analysis of incidence by treatment and mode of death yielded a Mantel-Haenszel estimate of the odds ratio of .79 with a Chi-square statistic of .06 ($p = .81$, $df = 1$), indicating that no difference in incidence existed between the exposed and sham-exposed animals.

TABLE 7. ATRIAL THROMBOSIS/NUMBER OF ANIMALS

		Age (months) at death					
		1-6	7-12	13-18	19-24	25-30	Total
Exposed:	Terminated	0/2	0/3	0/13	3/6	2/16	5/40
	Spontaneous	0/1	0/2	1/12	1/29	2/16	4/60
		0/3	0/5	1/25	4/35	4/32	9/100
Sham Exposed:	Terminated	0/1	0/3	0/12	1/1	5/18	6/35
	Spontaneous	0/2	1/5	2/11	1/34	1/13	5/65
		0/3	1/8	2/23	2/35	6/31	11/100

Foci of cellular alteration (syn: nodular hyperplasia) is described as a separate entity in this report and is rather arbitrarily separated from cortical adenoma even though the two lesions may be part of a continuous spectrum of disease (Strandberg, 1983). This separation of lesions is often of practical importance when they occur in bioassay studies. A somewhat arbitrary criterion is the absence of compression of adjacent cortical tissue in hyperplasia. This criterion is subjective, and terminology in the literature varies considerably. There are basically two schools of thought on the nomenclature; some individuals are reluctant to use "neoplasms" for anything but large or obviously invasive or metastatic lesions, thus the term "nodular hyperplasia" for the majority of cases. In contrast, the other school of thought holds that because one cannot, on histopathologic grounds, reliably differentiate hyperplastic lesions from benign tumors, all these nodular growths should be considered neoplastic and termed adenomas or (if they invade or metastasize) carcinomas. This variance in nomenclature and criteria makes it difficult to compare data derived from different studies.

Foci of cellular alteration, cortical adenomas, and adenocarcinomas are seen with increasing frequency in older rats (Boorman and Hollander, 1973; Anver, Cohen, Lattuada, and Foster, 1982). The foci of cellular alteration are roughly spherical collections of adrenal cortical cells that do not compress the surrounding cortical parenchyma. The cells closely resemble those of the surrounding cortex and possess round vesicular nuclei and pale eosinophilic cytoplasm that is often slightly vacuolated. Some foci may be formed of cells with highly vacuolated cytoplasm, while others consist of smaller cells with denser, more basophilic cytoplasm. Mitotic activity is low in all of these lesions.

A comparison of the incidence of various nonspecific foci of cellular alterations in the adrenal glands is presented in Table 8, arranged by treatment condition, age, and mode of death. The 2 x 2 analysis of incidence by treatment and mode of death yielded a Mantel-Haenszel estimate of the odds ratio of .61 with a Chi-square statistic of 1.85 ($p = .17$, $df = 1$), indicating that no difference in diagnostic incidence existed between the exposed and sham-exposed animals.

TABLE 8. ADRENAL CELLULAR ALTERATIONS/NUMBER OF ANIMALS

		Age (months) at death					
		1-6	7-12	13-18	19-24	25-30	Total
Exposed:	Terminated	0/2	0/3	5/13	4/6	14/16	23/40
	Spontaneous	0/1	1/2	4/12	12/29	12/16	29/60
		0/3	1/5	9/25	16/35	19/32	52/100
Sham Exposed:	Terminated	0/1	0/3	6/12	0/1	16/18	22/35
	Spontaneous	1/2	0/5	2/11	15/34	13/13	31/65
		1/3	0/8	8/23	15/35	29/31	53/100

Arteriosclerosis consisting of subintimal and medial calcification occurred in the intrapulmonary branches of the pulmonary artery, thoracic aorta, and testicular arterioles. Focal calcium deposition in the media of the arteries occurred without associated degenerative changes. It consisted of basophilic, amorphous deposits of calcium; no identifiable etiology was apparent and the lesions appeared relatively insignificant.

The incidence of arteriosclerosis is presented in Table 9, arranged by treatment condition, age, and mode of death. The 2 x 2 analysis of incidence by treatment and mode of death yielded a Mantel-Haenszel estimate of the odds ratio of .38 with a Chi-square statistic of 0.64 ($p = .42$, $df = 1$), indicating that no difference in incidence existed between the exposed and sham-exposed animals.

TABLE 9. ARTERIOSCLEROSIS INCIDENCE/NUMBER OF ANIMALS

		Age (months) at death					
		1-6	7-12	13-18	19-24	25-30	Total
Exposed:	Terminated	1/2	0/3	0/13	0/6	0/16	1/40
	Spontaneous	0/1	0/2	1/12	0/29	0/16	1/60
		1/3	0/5	1/25	0/35	0/32	2/100
Sham Exposed:	Terminated	0/1	0/3	0/12	0/1	0/18	0/35
	Spontaneous	0/2	0/5	1/11	4/34	0/13	5/65
		0/3	0/8	1/23	4/35	0/31	5/100

The incidence of testicular atrophy increased in older rats. The lesions consisted of seminiferous tubular degeneration, often with giant or bizarre-shaped cells present in the tubules. Dystrophic calcification occurred in some degenerating tubules, and periarteritis and arteriosclerosis of testicular arterioles often occurred in the atrophic testis.

The incidence of testicular atrophy is presented in Table 10, arranged by treatment condition, age, and mode of death. The 2 x 2 analysis of incidence by treatment and mode of death yielded a Mantel-Haenszel estimate of the odds ratio of 0.83 with a Chi-square statistic of 0.07 ($p = .79$, $df = 1$), indicating that no difference in incidence existed between the treatment conditions.

TABLE 10. TESTICULAR ATROPHY INCIDENCE/NUMBER OF ANIMALS

		Age (months) at death					
		1-6	7-12	13-18	19-24	25-30	Total
Exposed:	Terminated	0/2	0/3	0/13	2/6	3/16	5/40
	Spontaneous	0/1	0/2	0/12	5/29	5/16	10/60
		0/3	0/5	0/25	7/35	8/32	15/100
Sham Exposed:	Terminated	0/1	0/3	2/12	1/1	2/18	5/35
	Spontaneous	0/2	0/5	0/11	7/34	5/13	12/65
		0/3	0/8	2/23	8/35	7/31	17/100

Age-associated variability in the size of thyroid follicles, decreased amounts of pale colloid content, accumulation of basophilic debris and calcific concretions, and squamous metaplasia of the follicular epithelium represent atrophic changes in the thyroid gland. The incidence of thyroid atrophy is presented in Table 11, arranged by treatment condition, age, and mode of death. The 2 x 2 analysis of incidence by treatment and mode of death yielded a Mantel-Haenszel estimate of the odds ratio of 0.63 with a Chi-square statistic of 1.10 ($p = .29$, $df = 1$), indicating that no difference in incidence existed between the treatment conditions.

TABLE 11. THYROID ATROPHY INCIDENCE/NUMBER OF ANIMALS

		Age (months) at death					
		1-6	7-12	13-18	19-24	25-30	Total
Exposed:	Terminated	0/2	2/3	7/13	6/6	16/16	31/40
	Spontaneous	0/1	2/2	11/12	23/29	12/16	48/60
		0/3	4/5	18/25	29/35	28/32	79/100
Sham Exposed:	Terminated	1/1	1/3	10/12	1/1	16/18	29/35
	Spontaneous	2/2	3/5	9/11	29/34	13/13	56/65
		3/3	4/8	19/23	30/35	29/31	85/100

Several of the rats had microscopic cysts within the anterior pituitary gland that appear as irregular foci in which parenchymal cells are lost. These cysts are lined by the viable-appearing cells of the anterior pituitary. The cysts contain finely granular, eosinophilic proteinaceous material and occasional cellular remnants. This lesion represents a mild cystoid degeneration.

The incidence of pituitary cysts is presented in Table 12, arranged by treatment condition, age, and mode of death. The 2 x 2 analysis of incidence by treatment and mode of death yielded a Mantel-Haenszel estimate of the odds ratio of 1.90 with a Chi-square statistic of 2.65 ($p = .10$, $df = 1$), indicating no statistically significant evidence that this incidence is more likely to be present in an exposed than in a sham-exposed population.

TABLE 12. PITUITARY CYST INCIDENCE/NUMBER OF ANIMALS

		Age (months) at death					
		1-6	7-12	13-18	19-24	25-30	Total
Exposed:	Terminated	0/2	0/3	5/13	2/6	8/16	15/40
	Spontaneous	0/1	1/2	3/12	7/29	1/16	12/60
		0/3	1/5	8/25	9/35	9/32	27/100
Sham Exposed:	Terminated	0/1	0/3	3/12	0/1	4/18	7/35
	Spontaneous	0/2	0/5	3/11	3/34	3/13	9/65
		0/3	0/8	6/23	3/35	7/31	16/100

Chronic preputial adenitis is common in rats over 12 months of age (Ekstrom and Ewald, 1975; Anver, Cohen, Lattuada, and Foster, 1982). Glandular ducts are usually distended with inspissated secretions and necrotic debris. Suppurative foci and areas of granulomatous inflammation are often present, with opportunistic fecal flora usually cultured from the lesions. Some of the lesions appear to be secondary to cystic hyperplasia, while others appear to be a primary inflammatory lesion.

The incidence of preputial adenitis is presented in Table 13, arranged by treatment condition, age, and mode of death. The 2 x 2 analysis of incidence by treatment and mode of death yielded a Mantel-Haenszel estimate of the odds ratio of 1.40 with a Chi-square statistic of .67 ($p = .41$, $df = 1$), indicating no evidence that this incidence was more likely in either treatment condition.

TABLE 13. PREPUTIAL ADENITIS INCIDENCE/NUMBER OF ANIMALS

		Age (months) at death					
		1-6	7-12	13-18	19-24	25-30	Total
Exposed:	Terminated	0/2	0/3	9/13	0/6	3/16	12/40
	Spontaneous	0/1	2/2	2/12	5/29	3/16	12/60
		0/3	2/5	11/25	5/35	6/32	24/100
Sham Exposed:	Terminated	0/1	0/3	3/12	0/1	1/18	4/35
	Spontaneous	0/2	0/5	2/11	10/34	2/13	14/65
		0/3	0/8	5/23	10/35	3/31	18/100

With regards to the incidences presented in Tables 4-13, it was of interest to know if differences existed between treatment conditions relative to the severity of the four observed lesions--glomerulonephropathy, cardiomyopathy, thyroid atrophy, and pituitary cyst. These four lesions were evaluated because they were considered to have possible effects on the serum chemistry results. A summary for each diagnosis of interest is presented in Tables 14-17. The analyses were each based on a Chi-square evaluation of expected frequencies calculated from the appropriate tables, and each analysis indicated that no significant differences existed between treatment groups with respect to lesion grade distribution. The Chi-square statistic for the analysis of glomerulonephropathy was 7.78 ($p = .1$, $df = 4$); cardiomyopathy, 0.52 ($p = .97$, $df = 4$); thyroid atrophy, 4.15 ($p = .25$, $df = 3$); and pituitary cyst, 5.20 ($p = .16$, $df = 3$).

TABLE 14. GLOMERULONEPHROPATHY DIAGNOSES/NUMBER OF ANIMALS

Group:		Age (months) at death					
Mode of death		1-6	7-12	13-18	19-24	25-30	Total
Exposed:							
Terminated	minimal	0/2	2/3	3/13	0/6	1/16	6/40
	mild	0/2	0/3	6/13	1/6	2/16	9/40
	moderate	0/2	0/3	1/13	4/6	4/16	9/40
	marked	1/2	1/3	0/13	1/6	8/16	11/40
Spontaneous	minimal	0/1	1/2	4/12	3/29	0/16	8/60
	mild	0/1	0/2	3/12	6/29	1/16	10/60
	moderate	0/1	1/2	1/12	12/29	12/16	26/60
	marked	0/1	0/2	1/12	4/29	3/16	8/60
		1/3	5/5	19/25	31/35	31/32	87/100
Sham Exposed:							
Terminated	minimal	0/1	3/3	5/12	0/1	0/18	8/35
	mild	0/1	0/3	6/12	0/1	3/18	9/35
	moderate	0/1	0/3	1/12	0/1	5/18	6/35
	marked	0/1	0/3	0/12	1/1	10/18	11/35
Spontaneous	minimal	1/2	4/5	3/11	4/34	3/13	15/65
	mild	0/2	0/5	3/11	11/34	1/13	15/65
	moderate	0/2	0/5	2/11	17/34	6/13	25/65
	marked	1/2	0/5	3/11	1/34	2/13	7/65
		2/3	7/8	23/23	34/35	30/31	96/100

TABLE 15. CARDIOMYOPATHY DIAGNOSES/NUMBER OF ANIMALS

Group:		Age (months) at death					
Mode of death		1-6	7-12	13-18	19-24	25-30	Total
Exposed:							
Terminated	minimal	0/2	0/3	1/13	0/6	0/16	1/40
	mild	0/2	0/3	2/13	2/6	4/16	8/40
	moderate	1/2	1/3	0/13	0/6	1/16	3/40
	marked	0/2	0/3	0/13	0/6	0/16	0/40
Spontaneous	minimal	0/1	1/2	1/12	4/29	0/16	6/60
	mild	0/1	0/2	1/12	2/29	1/16	4/60
	moderate	0/1	0/2	0/12	3/29	1/16	4/60
	marked	0/1	1/2	0/12	1/29	0/16	2/60
		1/3	3/5	5/25	12/35	7/32	28/100
Sham Exposed:							
Terminated	minimal	0/1	0/3	1/12	0/1	0/18	1/35
	mild	0/1	0/3	2/12	0/1	3/18	5/35
	moderate	0/1	0/3	1/12	0/1	3/18	4/35
	marked	0/1	0/3	0/12	0/1	0/18	0/35
Spontaneous	minimal	0/2	1/5	2/11	2/34	0/13	5/65
	mild	0/2	0/5	2/11	6/34	0/13	8/65
	moderate	1/2	0/5	1/11	2/34	0/13	4/65
	marked	0/2	0/5	0/11	0/34	1/13	1/65
		1/3	1/8	9/23	10/35	7/31	28/100

TABLE 16. THYROID ATROPHY DIAGNOSES/NUMBER OF ANIMALS

Group:		Age (months) at death					
Mode of death		1-6	7-12	13-18	19-24	25-30	Total
Exposed:							
Terminated	minimal	0/2	1/3	1/13	1/6	3/16	6/40
	mild	0/2	1/3	6/13	3/6	9/16	19/40
	moderate	0/2	0/3	0/13	2/6	4/16	6/40
	marked	0/2	0/3	0/13	0/6	0/16	0/40
Spontaneous	minimal	0/1	0/2	0/12	3/29	0/16	3/60
	mild	0/1	0/2	6/12	11/29	7/16	24/60
	moderate	0/1	2/2	5/12	9/29	5/16	21/60
	marked	0/1	0/2	0/12	0/29	0/16	0/60
		0/3	4/5	18/25	29/35	28/32	79/100
Sham Exposed:							
Terminated	minimal	0/1	1/3	2/12	0/1	0/18	3/35
	mild	1/1	0/3	8/12	1/1	10/18	20/35
	moderate	0/1	0/3	0/12	0/1	6/18	6/35
	marked	0/1	0/3	0/12	0/1	0/18	0/35
Spontaneous	minimal	0/2	0/5	2/11	4/34	1/13	7/65
	mild	1/2	2/5	7/11	18/34	8/13	36/65
	moderate	1/2	1/5	0/11	7/34	4/13	13/65
	marked	0/2	0/5	0/11	0/34	0/13	0/65
		3/3	4/8	19/23	30/35	29/31	85/100

TABLE 17. PITUITARY CYST DIAGNOSES/NUMBER OF ANIMALS

Group:		Age (months) at death					
Mode of death		1-6	7-12	13-18	19-24	25-30	Total
Exposed:							
Terminated	minimal	0/2	0/3	3/13	0/6	5/16	8/40
	mild	0/2	0/3	2/13	2/6	3/16	7/40
	moderate	0/2	0/3	0/13	0/6	1/16	1/40
	marked	0/2	0/3	0/13	0/6	0/16	0/40
Spontaneous	minimal	0/1	1/2	2/12	3/29	1/16	7/60
	mild	0/1	0/2	1/12	2/29	0/16	3/60
	moderate	0/1	0/2	0/12	2/29	0/16	2/60
	marked	0/1	0/2	0/12	0/29	0/16	0/60
		0/3	1/5	8/25	9/35	10/32	28/100
Sham Exposed:							
Terminated	minimal	0/1	0/3	2/12	0/1	1/18	3/35
	mild	0/1	0/3	1/12	0/1	3/18	4/35
	moderate	0/1	0/3	0/12	0/1	0/18	0/35
	marked	0/1	0/3	0/12	0/1	0/18	0/35
Spontaneous	minimal	0/2	0/5	2/11	0/34	1/13	3/65
	mild	0/2	0/5	1/11	2/34	1/13	4/65
	moderate	0/2	0/5	0/11	1/34	1/13	2/65
	marked	0/2	0/5	0/11	0/34	0/13	0/65
		0/3	0/8	6/23	3/35	7/31	16/100

Neoplastic Lesions

A total of 192 neoplastic lesions were observed in the animals, with 83 unique combinations of organ and specific diagnosis. A summary of these combinations is presented in Table 18, indicating the total number of primary and metastatic malignancies and benign lesions observed for both the exposed and sham-exposed animals. The abbreviations of lesions are explained in Appendix C.

TABLE 18. NEOPLASTIC LESIONS PER ORGAN SYSTEM

Organ	Lesions	Exposed			Sham Exposed		
		B	P	M	B	P	M
Adrenal	Adenoma	0	0	0	1	0	0
	Carcinoma	0	0	0	0	1	0
	Cortical aden	10	0	0	10	0	0
	Cortical carc	0	3	0	0	0	0
	Leuk myelomono	0	0	0	0	0	1
	Malig lymph	0	0	1	0	0	0
	Pheochrom	7	0	0	1	0	0
Blood vessel	Hemangiosarc	0	1	0	0	0	0
Bone marrow	Leukemia	0	0	0	0	0	1
	Leuk myelomono	0	0	1	0	0	1
	Malig lymph	0	1	0	0	0	0
Brain	Leuk myelomono	0	0	0	0	0	1
	Malig lymph	0	0	2	0	0	0
Cerv. l node	Leuk myelomono	0	0	0	0	0	1
	Lymphocy lymph	0	0	0	0	1	0
	Malig lymph	0	0	0	0	0	1
Colon	Malig lymph	0	0	1	0	0	0
Duodenum	Leuk myelomono	0	0	1	0	0	0
	Malig lymph	0	0	1	0	0	0
	Sq cell carc	0	0	1	0	0	0
Epididymus	Sq cell carc	0	0	1	0	0	0
Eye	Leukemia	0	0	0	0	0	1
Heart	Leuk myelomono	0	0	1	0	0	1
	Malig lymph	0	0	1	0	0	0
	Neurinoma	1	0	0	2	0	0
Kidney	Leukemia	0	0	0	0	0	1
	Leuk myelomono	0	0	1	0	0	1
	Malig lymph	0	0	1	0	0	0
	Nephroblastoma	1	0	0	1	0	0
Liver	Adenoma	2	0	0	0	0	0
	Carcinoma	0	0	0	0	1	0
	Hepatoc adenom	1	0	0	0	0	0
	Leukemia	0	0	0	0	0	1
	Leuk myelomono	0	0	2	0	0	1
	Malig lymph	0	0	1	0	0	1
	Sq cell carc	0	0	1	0	0	0
Lung	Leukemia	0	0	0	0	0	1
	Leuk myelomono	0	0	1	0	0	0
	Malig lymph	0	0	1	0	0	0
Lymph node	Leuk myelomono	0	1	2	0	1	0
	Malig lymph	0	0	1	0	0	0
	Tran cell carc	0	0	1	0	0	0
Mesentery	Tran cell carc	0	0	1	0	0	0
Nasal cavity	Leukemia	0	0	0	0	0	1
Pancreas	Adenoma	0	0	0	1	0	0
	Islet-cel aden	1	0	0	1	0	0

TABLE 18. NEOPLASTIC LESIONS (continued)

Organ	Lesion	Exposed			Sham Exposed		
		B	P	M	B	P	M
Pancreas	Sq cell carc	0	0	1	0	0	0
Parathyroid	Malig lymph	0	0	1	0	0	0
Parotid SG	Leuk myelomono	0	0	1	0	0	0
Peritoneum	Liposarcoma	0	1	0	0	0	0
Pituitary	Adenoma	17	0	0	21	0	0
	Carcinoma	0	2	0	0	0	0
Preputial gland	Malig lymph	0	0	1	0	0	0
Skeletal muscle	Leuk myelomono	0	0	1	0	0	0
Skin	Aud seb sq car	0	1	0	0	0	0
	Basal cell carc	0	1	0	0	0	0
	Basal cell tum	1	0	0	0	0	0
	Keratoacanth	1	0	0	1	0	0
	Malig lymph	0	0	1	0	0	0
	Pilomatricoma	1	0	0	0	0	0
	Sebaceous aden	2	0	0	0	0	0
Spleen	Leuk myelomono	0	0	1	0	0	1
	Malig lymph	0	0	1	0	0	0
Stomach	Malig lymph	0	0	1	0	0	0
	Sq cell carc	0	1	0	0	0	0
	Sq cell papilloma	3	0	0	4	0	0
SubQ tissue	Fibroma	1	0	0	0	0	0
	Fibrosarc	0	1	0	0	0	0
	Lipoma	1	0	0	0	0	0
	Neurinoma	0	0	0	1	0	0
Testes	Int cl tum bn	1	0	0	0	0	0
	Sq cell carc	0	0	1	0	0	0
Thymus	Leuk myelomono	0	1	0	0	0	0
	Lymphocy lymph	0	1	0	0	0	0
	Malig lymph	0	0	0	0	1	0
Thyroid	Adenoma C-cell	10	0	0	9	0	0
	Carc C-cell	0	2	0	0	0	0
	Leukemia	0	0	0	0	0	1
	Malig lymph	0	0	1	0	0	0
Ureter	Malig lymph	0	0	1	0	0	0
Urin/bladder	Tran cell carc	0	1	0	0	0	0
	Tran cell papiloma	1	0	0	0	0	0
Zymbal's gland	Leukemia	0	0	0	0	0	1
Total		62	18	36	53	5	18

Note: This table lists neoplastic lesions found per organ system. These lesions may be benign (B), a primary malignancy (P), or a metastatic malignancy (M) arising from a primary malignancy in another organ system (i.e., a malignant neoplasm may occur as a metastatic malignancy in many organs of a single animal, but as a primary malignancy in only one organ system of an animal).

Only two types of tumors were present in rats younger than 12 months--kidney nephroblastoma and stomach papillary carcinoma. After 18 months the incidence of neoplasms increased rapidly, especially those involving the endocrine system.

In an initial analysis of the neoplastic lesions, 4-way tables were constructed, factoring age at death, treatment condition, mode of death, and organ. The data were sparse, however, so few, if any, conclusions could be drawn from the tables. The tables were then collapsed with respect to organs; only the presence of a lesion was of concern (Table 19); no attention was given to the area of occurrence. Benign neoplasms were treated as "incidental"--an accidental finding at death, not a contributing cause. Metastatic lesions occurred too seldom for meaningful comparison, so they were ignored for this analysis.

Following the practice used in previous studies, age at death was divided into 6-month partitions as follows: 1-6, 7-12, 13-18, 19-24, and 25-30 (e.g., 1-6 indicates age from 1 month through 6 months of age).

TABLE 19. INCIDENCE OF BENIGN NEOPLASMS AT DEATH

Age (mo)	Benign neoplasms	No. of animals	
		Exposed	Sham
1-6	Yes	0	0
	No	3	3
7-12	Yes	0	3
	No	5	5
13-18	Yes	1	5
	No	24	18
19-24	Yes	16	11
	No	19	24
25-30	Yes	22	19
	No	10	12

From this series of 2 x 2 tables, the Mantel-Haenszel (M-H) estimate of the odds ratio was 1.04. The Chi-square statistic, which tests whether or not the relative risk is 1, was .001 ($p = .97$, $df = 1$); therefore, we find no evidence that either group had an excess of benign lesions.

A similar set of 2 x 2 tables was prepared for primary malignant neoplastic lesions and is presented in Table 20.

TABLE 20. INCIDENCE OF PRIMARY MALIGNANT LESIONS AT DEATH

Age	Primary malignant lesions	No. of animals	
		Exposed	Sham
Age considered			
1-6	Yes	0	0
	No	3	3
7-12	Yes	0	0
	No	5	8
13-18	Yes	2	2
	No	23	21
19-24	Yes	9	1
	No	26	34
25-30	Yes	7	2
	No	25	29
Age not considered			
	Yes	18	5
	No	82	95

When all age categories for the primary malignant lesions were considered, the M-H estimate of the odds ratio was 4.27 and the Chi-square statistic was 7.66 ($p = .006$, $df = 1$). With the first three age categories combined and the analysis repeated, the M-H statistic was 4.38 and the Chi-square statistic was 7.9 ($p = .005$, $df = 1$). When the first four age categories were collapsed (leaving two categories--1-24 and 25-30 mo), the M-H statistic was 4.47 and the Chi-square was 6.97 ($p = .008$, $df = 1$).

When age at death was ignored completely, the M-H estimate of the relative risk was 4.46 and the Chi-square was 8.00 ($p = .005$, $df = 1$). It is interesting that the estimate of the odds ratio and the Chi-square statistic are both insensitive to the way the data were grouped with respect to age at death.

A survival-type analysis also was done using time of death as the endpoint if a primary malignant lesion were present. If no primary malignant lesions were found, time of death was ignored. From that analysis, the log-rank statistic is 7.63 with a p-value of .006. This analysis suggests that the primary tumors occurred earlier in the exposed group than in the sham exposed.

To summarize the above results, primary malignancies are somewhat more likely to be present in exposed animals than in the sham exposed. This should not be considered as some artifact of the data, since different analyses led to similar results.

Parasitology

All of the rats were consistently negative for ectoparasites. Of the animals in the colony, 29 (approximately 15%) had a low level of infestation with Syphacia muris. Pinworms are common in most rat colonies and even with SPF colonies it is often difficult to eliminate them or to keep them out. The level of infestation in this colony was low because each animal had its own individual experimental cage and holding cage, and only that animal was placed in these cages during its lifetime. The parasites may have entered the colony on the cages after cleaning, because the cages, made of polypropylene, could not be autoclaved but were sanitized with 83°C water. Histologically, no lesions could be attributed to these nematodes, indicating that low numbers of these worms do not appear to have any significant effect on the longevity of the animal or the morphological features of the cecum and colon.

Microbiology

During the 25-month experimental period the defined microflora was altered by the sporatic occurrence of *Proteus sp.* (*mirabilis*, *rettgeri*, and *vulgaris*), *Staphylococcus epidermidis*, *Neisseria sp.*, *Escherichia coli*, and *Klebsiella sp.* These intestinal flora may become opportunistic organisms in lesions such as chronic preputual adenitis or wound infections. *Mycoplasma sp.* was not isolated, either by culture or serology, from any animal during the study.

Serology

Monitoring of the animals failed to reveal any significant titers to any of the rodent viruses or to mycoplasma. The animals were maintained free of any specific pathogens throughout the study, and there was no concern about an underlying disease affecting the experimental results.

DISCUSSION

This phase of the RFR bioeffects program examined grossly and histologically the 43 interim- and final-sacrifice animals and the 157 animals that died spontaneously during the study. Evaluation of the cumulative survival curves for both the exposed and sham-exposed animals revealed that the median survival time for the exposed animals was 688 days and for the sham-exposed animals, 663 days. Despite subtle differences in the survival curves in the early and late stages of the study, statistical analysis concluded that no significant effect existed during any phase of the life span of the animals. A decrease in the mean survival age of both treatment groups, as compared to those reported in the literature, is due to removal of animals for two experimental groups--the interim and final kills.

Statistical evaluation indicated no association between a specific cause of death and the treatment condition; however, for cause of death due to urinary tract blockage, there is some indication that the survival times are longer in the exposed animals.

The documentation of morphological lesions resulted in 2184 observations of pathological changes in the 200 animals examined. The nonneoplastic lesions comprised 1992 of the observations; with 217 unique combinations of organs and lesions. The neoplastic lesions accounted for 192 of the observations, with 83 unique combinations of organs and type of neoplasms.

Chronic glomerulonephropathy is the most frequent cause of death and one of the most consistently encountered lesions. Statistical analysis indicates that glomerulonephropathy is less frequently observed in the exposed than in the sham-exposed animals. Analysis of the other nonneoplastic lesions does not indicate that the specific lesions are more likely in either treatment condition.

To detect a progressive development of the chronic glomerulonephropathy, the severity of the lesions also was evaluated. This analysis revealed no significant differences between the treatment condition and the severity of the lesions.

The neoplastic lesions were identified as benign or malignant, with the malignant lesions classified as primary or metastatic. The incidence of neoplastic lesions corresponds to that reported for this strain of rat; only two tumors were present in rats younger than 12 months, and the incidence rapidly increased after 18 months of age. The endocrine system had the highest incidence of neoplasia in the aging rats, as is to be expected in this experimental animal.

The low incidence of neoplasia with no increase in any specific organ or tissue required the data to be collapsed and statistically evaluated with respect only to occurrence of the neoplasm, with no attention given to the area of occurrence. This analysis indicated that neither group had an excess of benign lesions. There is statistical evidence that the mean number of primary malignancies was higher in the exposed animals than in the sham exposed, but the biological significance of this difference is reduced by several factors. First, detection of this difference required the collapsing of sparse data without regard for the specific type of malignancy or tissue of origin. Also, when the incidence of the specific primary malignancies in the exposed animals is compared with specific tumor incidence reported in the literature, our exposed animals had an incidence similar to that of untreated control rats of the same strain, maintained under similar SPF conditions (Anver, Cohen, Lattuada, and Foster, 1982). It is important to note that no single type of primary malignancy was enhanced in the exposed animals. From the standpoint of carcinogenesis, benign neoplasms have considerable significance under the assumption that the initiation process is similar for both benign and malignant tumors. The fact that treatment groups showed no difference in benign tumor incidence is an important element in defining the promotion and induction potential of microwave radiation for carcinogenesis. The collapsing of sparse data without regard for tissue origin is useful in detecting possible statistical trends, and the finding here of excess primary malignancies in the exposed animals is provocative; however, when this single finding is considered in the light of other parameters evaluated, it is questionable if the statistical difference reflects a true biological activity (Ward, 1983). No meaningful statistical analysis could be made of metastatic neoplasms because of their low incidence.

To standardize the experimental animals as much as possible, the exposed and sham-exposed animals were housed under identical conditions and subjected to identical diet, handling, husbandry, lighting, air change, and sample-collection procedures. The animals were also monitored for any parasitic, bacterial, mycoplasmal, or viral agents during the 25-month experimental period. No significant infections occurred that would complicate or produce erroneous results in the gross or histopathological evaluation of the experimental animals.

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APPENDIX A

NECROPSY TECHNIQUE FOR MALE RATS

I. Introduction

Rapid diagnosis of small laboratory animal diseases and lesions is difficult. Because of the small size of the animals and because of the similarity of responses to a variety of pathogens, toxic agents, and degenerative changes, a definitive diagnosis depends on meticulous use of gross, microscopic, clinical, serological, and bacteriological examinations.

II. Preliminary Observations

A. Before beginning the necropsy, fill out the animal pathology record.

B. Outline the clinical history (history of illness, appetite, water consumption, position of animal at time of death, etc.).

C. Record the clinical diagnosis (renal failure, neoplasia, etc.).

III. External Examination

A. Note general appearance of carcass (state of nutrition, general conformation, etc.).

B. Look for evidence of postmortem changes (bloating, rigor, discoloration, etc.).

C. Examine eyes, ears, nostrils, mouth, integument, foot pads, genitalia, anus, sheath--for color of mucous membranes, discharges, enlargements, wounds, hair coat and skin changes, inflammation, external parasites, etc.

D. Check breed, sex, age, and weight.

IV. Opening the Body Cavities

A. Prepare for specific procedures.

1. Wet the animal's down to prevent hair from contaminating the internal organs.

2. Place the animal in dorsal recumbency with the head away from the examiner or to your right hand.

3. Set out a jar of fixative, a cork cutting board, knife, forceps, scissors, and scales if protocol requires organ weights. Equipment for culturing and collecting laboratory samples should be available.

4. Remember that the entire carcass, including all systems and organs, must be carefully examined. Lesions may appear anywhere, and care should be taken to expose and examine all lesions. Protect lesions from contamination for possible culture. Examine carefully both of the paired organs. Incise the left organ longitudinally; the right, transversely.

B. Incise the skin at axilla.

1. Cut the coxofemoral articulation and continue a midline skin incision anteriorly to the ramus of the mandible.
2. Dissect the skin dorsally on both sides.
3. Examine the prescapular lymph nodes and adjacent subcutaneous connective tissue.
4. Examine the salivary glands and cervical lymph nodes.
5. Examine the preformal lymph nodes, muscles, head of the femur, and exposed joint cavity.

C. Examine the mammary glands (may be found in some male rats).

D. Open prepuce; examine penis but leave attached to symphysis.

E. Open abdomen by a midline incision; cut diaphragm and listen for inrush of air.

F. Sever the ribs on both sides at the sternum and remove the chest plate, thus exposing the entire thoracic cavity.

1. Save a piece of sternum for bone marrow study.
2. Dissect free one rib and break if possible.

V. Gross Examination of the Thoracic and Abdominal Cavities

A. Without disarranging the viscera to any extent, look for transudates, exudates, hemorrhage, etc. Open the heart sac and examine the pericardial sac contents.

B. Examine for adhesions, displacements, absence of organs, size and symmetry of organs <u>in situ</u>.

C. Observe vagus and phrenic nerves.

VI. Removal and Examination of the Thoracic Viscera

A. Inspect heart and pericardium *in situ*. Check for pericardial fluid before disturbing thoracic viscera (Section V.A). Examine fluid and measure or estimate its volume.

B. Observe mouth, pharynx, teeth.

C. Remove thoracic viscera.

1. Cut along lingual surface of both sides of the mandible. Loosen the tongue and pull it down between the rami. Disarticulate the hyoid bones, tongue, and larynx. Incise the soft palate anterior to the tonsils so that the tonsils and adjacent pharynx will remain attached to the larynx and tongue. (If required, take nasopharyngeal cultures with brain heart infusion broth.)
2. Grasping the tongue and pulling upward, dissect the trachea, esophagus, and carotid blood vessels from the muscles or the neck posteriorly to the thoracic inlet.
3. Continue removing the thoracic viscera by dissecting the aorta and mediastinum free from the dorsal thoracic wall to the diaphragm.
4. Grasping the trachea, lungs, and aorta and while pulling forward, sever the esophagus, posterior vena cava, and aorta immediately anterior to the diaphragm. Cutting the remainder of the mediastinal and pleural attachments will free the thoracic viscera from the thoracic cavity.

D. Examine the respiratory tract.

1. Open and examine the esophagus. Leave the esophagus attached to the respiratory tract at one point.
2. Examine the tonsils, thyroids, and parathyroids. Note size, consistency, etc.
3. Examine the bronchial lymph nodes by palpating and incising.
4. Observe and palpate lungs carefully for consolidation, emphysema, or other abnormal consistency.
5. Perfuse the lungs with 15% buffered formalin and suspend in formalin. (Sections and dissection are done after fixation.)

6. Open the larynx, trachea, bronchi, and small bronchioles.
   a. Look for exudate, hemorrhage, foreign bodies, or lung worms in bronchial tree.
   b. Examine areas of consolidation and other abnormalities in the lungs by incising them. Make multiple transverse sections.
   c. Check the pulmonary arteries for thrombi and parasites.
   d. Trim and embed lungs for horizontal sectioning.

E. Examine the heart and major vessels.
   1. Examine the heart while attached to the lungs.
   2. Observe any disproportion of parts (dilation, hypertrophy, or anomalies).
   3. Examine the surface of the heart, coronary vessels, and great vessels (remove uncontaminated blood with sterile syringe for culture and serology, if indicated).
   4. Open the heart while attached to the lungs and larger vessels. Place the tongue with the trachea to your left and the apex towards you, then the pulmonary artery will be visible between the two auricles. The right ventricle faces up. Make an incision, using scissors, into the pulmonary artery, and open the right ventricle by cutting towards the apex parallel to the interventricular septum. Continue the pulmonary artery incision into the lung as far as possible. Following the direction of blood flow, examine all valves and surfaces (post vena cava to pulmonary artery).
   5. With the left ventricle turned toward you, cut through the wall of the left ventricle and atrium to expose the chambers. Examine the left atrioventricular valve; include the posterior cusp which has been cut, the atrium, the pulmonary veins. Cut through the anterior cusp into the aorta and examine aortic valves.
   6. Examine the vessels and septa for anomalies.
   7. Examine the endocardium and then make multiple slices through the septum and ventricular walls to examine the myocardium and coronary vessels. Check the papillary muscles carefully for lesions.
   8. Open and examine the aorta and vena cava as far as possible.

VII. Examination of the Abdominal Aorta

A. Dissect through the root of the diaphragm and the subvertebral tissues to expose the abdominal aorta.

B. Open the length of the aorta with scissors and carefully examine for thrombi, aneurysms, intimal plaques, etc.

VIII. Removal and Examination of Abdominal Viscera

A. Cut symphysis - to complete removal.

B. Culture liver and spleen if indicated (direct culture or submit section to laboratory in a sterile petri dish).

C. Examine pancreas - leave attached to duodenum.

D. Examine the liver.

1. Examine the peritoneal surface for fibrosis and adhesions.
2. Note the size, shape, color, and consistency. Check all surfaces.
3. Palpate and incise all lobes of the liver. Look for necrosis, fibrosis, and abscesses.

E. Examine the adrenal glands before the kidneys are disturbed and then remove them attached to the kidneys when these are removed. Cut the adrenals in cross-section and note cortical-medullary ratio. In a very small animal the cross-sections are made after fixation.

F. Examine the stomach and intestines to the rectum.

1. Free the intestine from the mesentery and observe the lymph nodes.
2. Postpone examination of the gastrointestinal tract until last so that instruments and working area are not contaminated.

G. Remove genitourinary organs as a unit.

1. Cut each kidney longitudinally in half from the concave surface to the cortex.
2. Strip off capsule and examine one side of the kidney surface. Note ease with which capsule comes off. Make transverse cuts on the right kidney and longitudinal cuts on the left one.

3. Open and examine the ureters, bladder, and urethra. Inspect all mucosal and serosal surfaces.
4. Observe male accessory sex organs; note size, consistency, etc.

IX. Removal and Examination of Other Systems

A. Examination of the joints

1. Open five joints in a routine necropsy; one humeroscapular, one coxofemoral, and the occipitoatloid joints. To open the stifle joint, cut the straight patellar ligament 1/3 the way proximally to the tibial tuberosity and medial to the trochlea of the femur and reflect the patella.
2. Observe synovia, articular surfaces, articular cartilages, and synovial membranes.

B. Examination of the muscular system

1. Examine and incise the muscles of various parts of the body. These should include the serratus ventralis, the gracilis and the longissimus dorsi psoas muscles, and the diaphragm.
2. Incise the muscles of the abdominal wall liberally.
3. Check for size, development, color, etc.

C. Examination of the skeletal system

1. Examine body for broken bones or healed fractures.
2. For bone marrow, examine the center of the sternum.

D. Examination of eyes

1. Examine both eyes. Look for corneal opacities, cataracts, lens displacements, neoplasia, etc.
2. Leave eyes in skull for decalcification and histological examination within orbit along with harderian and lacrimal glands.

E. Examination of the central nervous system

1. Remove the brain.
   a. Transect the cord after opening the ventral portion of the occipitoatloid articulation to expose the spinal cord.
   b. Reflect the skin and muscles of head and examine skull for trauma.
   c. Disarticulate head at occipitoatloid articulation.

d. Remove the calvaria with heavy scissors by cutting from the foramen magnum.
e. Examine, carefully incise, and remove dura mater from the dorsal part of the brain.
f. Carefully clip the cranial nerves and allow the brain to slip out.
g. Remove the pituitary gland by cutting diaphragmatic sella on both sides, clipping bony projection posterior to the gland, and cutting soft tissue about the gland proper with scissors.
h. If no central nervous system disturbance has been observed clinically, incise the brain transversely (slices every centimeter) and look for lesions. If a disturbance has been observed clinically, preserve the brain in formalin intact.

2. Remove spinal cord (only when indicated).
a. Remove the ribs from both sides of the thorax and the skin, muscles, and limbs from the left side and back.
b. Place the back and sacrum with the spinous processes of the vertebrae up.
c. Cut through arches of vertebrae in each side of spinous processes and remove the bone to expose the entire cord.
d. Cut through nerve roots on each side of cord and remove entire cord.
e. The spinal cord is preserved in formalin intact.

F. Examination of nasal cavities: Make a transverse incision through facial bones just posterior to canine teeth and examine the turbinates.

G. Examination of auditory structure: Make a transverse incision through the middle and inner ear and examine for infection (submit to histology laboratory for decalcification and sectioning).

H. Examination of the gastrointestinal tract

1. Open the stomach along the greater curvature (the esophagus has been opened).

a. Observe the serosal and mucosal surface.

b. Examine for hemorrhage, parasites, foreign bodies, and any abnormal ingesta.

2. Open the small intestine. Observe all surfaces and ingesta.

3. Open the cecum and colon back to the anus and examine carefully.

4. Take representative sections of the GI tract.

I. Other incisions and procedures necessary to expose additional lesions or suspected lesions in the remainder of the carcass

J. Records and sections

1. Make a careful postmortem record.

2. Preserve all lesions and special tissues in 10% aqueous formalin solution (tissue slices not over 0.5-1.0 cm thick and with at least 10 times the volume of formalin solution as tissue).

3. Use only objective, descriptive terms for pathological descriptions. Examples: They are "pinpoint red spots," not "petechiae." It is a "semisolid round 2-cm nodule of glistening greyish appearance that cuts easily, located at the costochondral junction of the 9th rib and projecting into the pleural cavity," not a "tumor on the ribs." In parentheses, you may use a pathological term such as "ulcer" or "infarct" if you think your description is lacking. Remember to describe location, color, size, shape, and consistency, using correct anatomical, physiological, and other scientific terms.

APPENDIX B. AGE; MODE OF DEATH; AND TOTAL NUMBER OF NONNEOPLASTIC AND BENIGN, PRIMARY, AND METASTATIC NEOPLASTIC LESIONS IN EACH ANIMAL AS A FUNCTION OF EXPOSURE CONDITIONS

(Term. = terminated animals; Spon. = spontaneous deaths)

Exposed							Sham Exposed					
Age (Days)		Nonneoplastic lesions	Neoplastic lesions			Rat No.	Age (Days)		Nonneoplastic lesions	Neoplastic lesions		
Term.	Spon.		B	P	M		Term.	Spon.		B	P	M
99		4	0	0	0	C 14						
	99	4	0	0	0	E 1						
						G 8		131	6	0	0	0
						F 18	139		4	0	0	0
141		6	0	0	0	I 7						
						J 9		182	6	0	0	0
	188	7	0	0	0	E 16						
						H 20		220	4	1	0	0
						G 9	227		3	0	0	0
247		4	0	0	0	F 5						
						J 12		248	6	1	0	0
						E 9		253	5	1	0	0
						I 18		281	4	0	0	0
						D 17	308		8	0	0	0
309		7	0	0	0	H 16						
						B 13	311		4	0	0	0
						D 4		335	11	0	0	0
	347	17	0	0	0	B 10						
351		12	0	0	0	C 6						
	366	11	0	0	0	G 15						
	393	9	0	0	0	D 6						
						G 13		394	15	0	0	0
						H 3		418	9	0	0	0
						F 9		425	8	0	0	0
	431	7	0	0	0	I 11						
	438	10	0	1	0	H 5						
448		10	0	0	0	A 5						
						A 8	448		6	0	0	0
						B 12	448		11	0	1	8
448		13	0	0	0	B 16						
						B 17	448		7	0	0	0
448		4	0	0	0	C 1						
						C 8	448		5	0	0	0
448		3	0	0	0	E 5						
448		3	0	0	0	E 7						
						E 13	448		5	0	0	0
448		7	0	0	0	F 1						
						F 8	448		5	0	0	0
448		5	0	0	0	F 15						

Exposed							Sham Exposed					
Age (Days)		Nonneo-plastic lesions	Neoplastic lesions			Rat No.	Age (Days)		Nonneo-plastic lesions	Neoplastic lesions		
Term.	Spon.		B	P	M		Term.	Spon.		B	P	M
						G 12	448		7	0	0	0
448		10	0	0	0	G 19						
448		10	0	0	0	H 6						
						H 8	448		6	0	0	0
						J 2	448		5	1	0	0
						J 3	448		6	0	0	0
448		7	0	0	0	J 14						
	452	11	0	0	0	D 5						
	457	18	0	0	0	A 10						
						F 3		457	9	1	0	0
						I 13		471	12	0	0	0
						C 13		476	7	1	0	0
						D 18		488	8	0	0	0
						B 3		501	5	0	0	0
505		9	0	1	0	G 5						
	506	5	0	0	0	J 7						
	507	3	0	0	0	D 16						
	508	9	0	0	0	I 14						
						J 20		516	8	0	0	0
						G 2	518		10	0	0	0
519		8	0	0	0	I 15						
						E 12	520		10	0	0	0
	522	9	0	0	0	F 6						
	526	11	0	0	0	I 19						
						H 12		534	4	1	1	2
	540	5	2	0	0	A 16						
541		10	0	0	0	A 11						
						J 17		546	7	1	0	0
	552	11	0	0	0	D 10						
	552	9	0	1	0	G 11						
	552	9	0	0	0	D 14						
						J 18		557	10	0	0	0
	559	13	0	0	0	F 10						
	562	6	0	0	0	B 1						
						C 4		562	9	0	0	0
						G 18		563	9	0	0	0
						F 17		569	8	0	0	0
						I 4		569	10	0	0	0
	569	3	0	1	17	J 5						
						A 4		577	7	0	0	0
						G 3		577	12	0	0	0
	580	8	0	0	0	D 1						
						A 13		586	7	0	0	0
	587	12	1	0	0	A 1						
589		9	1	1	0	J 11						
	590	8	1	1	2	B 19						

Exposed							Sham Exposed					
Age (Days)		Nonneo-plastic lesions	Neoplastic lesions			Rat No.	Age (Days)		Nonneo-plastic lesions	Neoplastic lesions		
Term.	Spon.		B	P	M		Term.	Spon.		B	P	M
	593	11	0	0	0	H 14						
						H 9		594	9	0	0	0
						E 4		597	10	1	0	0
						J 13		597	14	0	0	0
598		6	0	0	0	G 14						
						I 9		601	9	0	0	0
	605	10	1	0	0	I 5						
						I 8		606	10	0	0	0
						H 17		609	9	0	0	0
	613	9	0	1	6	H 15						
						C 2		615	15	1	0	0
						H 4		619	10	1	0	0
						F 20		622	6	0	0	0
						B 8		625	15	3	0	0
	626	11	2	0	0	J 19						
631		15	0	0	0	G 10						
	634	8	0	0	0	D 19						
						B 18		637	7	1	0	0
	637	14	1	0	0	I 1						
	638	10	0	0	0	B 11						
	639	8	1	0	0	E 11						
						G 20		646	8	0	0	0
						J 4		648	11	1	0	0
	650	9	1	1	5	H 10						
						F 4		653	18	0	0	0
	654	15	0	0	0	H 7						
	656	4	2	0	0	C 7						
						D 3		658	11	0	0	0
	661	12	3	0	0	E 6						
						E 8		661	12	0	0	0
						A 9		663	16	1	0	0
663		14	1	0	0	F 14						
	664	12	0	0	0	C 16						
	667	5	0	0	0	D 7						
	669	12	1	1	0	C 11						
673		19	1	1	0	E 14						
						D 13		680	6	0	0	0
						F 2		682	9	1	0	0
	688	11	0	0	0	C 10						
						E 3		689	11	0	0	0
						E 17		691	9	1	0	0
						C 3		706	15	0	0	0
	706	12	0	0	0	I 10						
						F 13	709		11	0	0	0
710		14	0	0	0	I 16						
						G 4		715	9	0	1	0

Exposed							Sham Exposed					
Age (Days)		Nonneo-plastic lesions	Neoplastic lesions			Rat No.	Age (Days)		Nonneo-plastic lesions	Neoplastic lesions		
Term.	Spon.		B	P	M		Term.	Spon.		B	P	M
						B 2		717	16	0	0	0
						I 12		718	9	1	0	0
719		19	1	0	0	C 15						
						H 18		722	13	1	0	0
	725	12	1	0	0	F 11						
	726	12	1	1	0	D 15						
						E 2		736	8	1	0	0
						H 2	736		14	0	0	0
	736	15	1	0	0	G 1						
						I 2		737	11	0	0	0
	737	10	1	0	0	J 16						
						A 20		739	14	0	0	0
	739	15	1	0	0	J 10						
						C 20		741	7	3	0	0
						I 20	743		10	1	0	0
744		17	0	0	0	H 11						
747		10	3	1	6	B 6						
	748	9	0	0	0	J 1						
	750	8	0	0	0	B 5						
						C 12		750	10	1	1	8
						D 12	751		22	3	0	0
						E 18	753		12	0	0	0
	753	13	1	0	0	F 19						
	757	12	1	1	0	H 1						
	759	14	1	0	0	I 6						
759		10	0	0	0	J 15						
	760	15	0	0	0	B 15						
	761	7	1	0	0	F 7						
	762	16	0	1	0	E 10						
						I 3		765	7	2	0	0
						A 3	770		21	0	0	0
	771	7	0	0	0	F 16						
						A 17		775	10	0	0	0
						F 12		781	11	2	0	0
						D 20		783	15	1	0	0
	785	16	1	0	0	A 15						
	790	10	0	0	0	A 7						
						B 9		791	14	1	1	0
						I 17	794		15	0	0	0
	795	11	1	1	0	B 14						
						D 8	803		13	1	0	0
						A 12		804	6	2	0	0
						E 20		804	15	1	0	0
	804	10	1	1	0	G 6						
						A 18		806	16	2	0	0
	806	14	1	1	0	E 19						

Exposed						Sham Exposed				
Age (Days) Term. Spon.	Nonneoplastic lesions	Neoplastic lesions B	P	M	Rat No.	Age (Days) Term. Spon.	Nonneoplastic lesions	Neoplastic lesions B	P	M
					A 2	811	7	0	0	0
811	11	0	0	0	A 6					
811	12	3	1	0	A 14					
811	7	3	0	0	A 19					
					B 4	811	6	0	0	0
811	11	2	0	0	B 7					
					B 20	811	5	2	0	0
811	13	2	0	0	C 5					
					C 9	811	16	0	0	0
					C 17	811	13	0	0	0
					C 18	811	9	1	0	0
811	9	0	0	0	C 19					
					D 2	811	8	2	0	0
					D 9	811	9	2	0	0
811	17	2	0	0	D 11					
811	17	1	0	0	E 15					
811	10	2	0	0	G 7					
811	12	5	0	0	G 16					
					G 17	811	12	1	0	0
					H 13	811	9	3	0	0
811	14	4	0	0	H 19					
811	18	2	0	0	J 6					
					J 8	811	11	0	0	0
Totals	1030	62	18	36			962	53	5	18

APPENDIX C. LESION GLOSSARY

Lesion	Abbreviation (Text Table 18)
acute hemorrhagic inflammation	
acute inflammation	
acute necrotic inflammation	
adenoma	
adnexal gland cyst	
alveolar macrophage	
aneurysm	
angiectasis	
arteriosclerosis	
atelectasis	
atrophy	
atrophy fibrosis calcification	
auditory sebaceous gland squamous carcinoma	Aud seb sq car
autolysis	
basal cell carcinoma	Basal cell carc
basal cell tumor	Basal cell tum
basophilic bodies	
benign interstitial cell tumor	Int cl tum bn
bile duct ectasia	
bile duct hyperplasia	
biliary cyst	
c-cell adenoma	Adenoma C-cell
c-cell carcinoma	Carc C-cell
c-cell hyperplasia	
calcification	
calculus	
carcinoma	
cardiomyopathy	
cartilaginous foci	
cartilaginous metaplasia	
chronic diffuse inflammation	
chronic focal inflammation	
chronic inflammation	
chronic progressive glomerulonephropathy	
chronic suppurative inflammation	
cirrhosis	
coagulation necrosis	
congestion	
cortical adenoma	Cortical aden
cortical carcinoma	Cortical carc
cryptorchid	
cyst	
cystic degeneration	
cystic ducts	
cystic hyperplasia	
cytoplasmic vacuoles	
degenerative vacuolar encephalopathy	
degeneration	

Lesion	Abbreviation
diffuse hyperplasia	
edema	
enterolithiasis	
epidermal inclusion cyst	
extramedullary hematopoiesis	
fat necrosis	
fatty degeneration	
fatty infiltration	
fibroma	
fibrosarcoma	Fibrosarc
fibrosis	
focal granulomatous inflammation	
focal hyperplasia	
foci of cellular alteration	
giant cells	
gliosis	
hemangiosarcoma	Hemangiosarc
hematoidin pigment	
hematopoiesis	
hemorrhage	
hemosiderin pigment	
hepatocellular adenoma	Hepatoc adenom
hyaline degeneration	
hydronephrosis	
hyperkeratosis	
hyperplasia	
hypertrophy	
hypoplasia	
interstitial pneumonia	
islet-cell adenoma	Islet-cel aden
keratin cyst	
keratitis	
keratoacanthoma	Keratocanth
kyphosis, scoliosis	
leukemia	
lipoma	
liposarcoma	
liquefactive necrosis	
lymphocytic infiltration	
lymphocytic lymphoma	Lymphocy lymph
lymphoid hyperplasia	
malignant lymphoma	Malig lymph
medial calcification	
megaesophagus	
membranous glomerulonephritis	
mucoid degeneration	
myelomonocytic leukemia	Leuk myelomono
necrosis	
nephroblastoma	
neurinoma	
nodular hyperplasia	

Lesion	Abbreviation
papillary carcinoma	
parasite	
periarteritis	
pheochromocytoma	Pheochrom
pigmentation	
pilomatricoma	
porphyrin pigment	
proteinaceous plug	
proteinaceous calculus	
reticuloendothelial hyperplasia	
rupture	
sebaceous adenoma	Sebaceous aden
sinusoidal histiocytosis	
sperm granuloma	
squamous cell papilloma	Sq cell papilloma
squamous cell carcinoma	Sq cell carc
telangiectasis	
thrombosis	
transitional cell carcinoma	Tran cell carc
transitional cell papilloma	Tran cell papiloma
verminous arterial plexus	

APPENDIX D. TOTAL NUMBER OF NONNEOPLASTIC LESIONS IN EACH ORGAN AS A FUNCTION OF EXPOSURE

Organ	Lesion	Exposed	Sham
Acc. Genital	Chronic focal inflammation	0	1
	Chronic inflammation	4	1
	Chronic suppurative inflammation	1	1
	Focal granulomatous inflammation	1	0
Adrenal	Congestion	1	0
	Cyst	1	1
	Foci of cellular alteration	79	76
	Hemorrhage	1	0
	Nodular hyperplasia	3	2
Blood Vessel	Acute inflammation	1	0
	Angiectasis	1	0
	Arteriosclerosis	2	5
	Calcification	3	2
	Hemorrhage	1	0
	Hypertrophy	3	2
	Medial calcification	14	11
	Periarteritis	17	12
	Telangiectasis	1	0
Brain	Aneurysm	0	1
	Basophilic bodies	1	1
	Degenerative vacuolar encephalopathy	7	3
	Gliosis	0	1
	Hemorrhage	3	0
	Hemosiderin pigment	1	1
	Liquefactive necrosis	1	0
	Thrombosis	1	0
Cecum	Chronic inflammation	1	0
	Parasite	2	1
Cerv. L Node	Acute inflammation	1	0
	Chronic suppurative inflammation	0	1
	Congestion	0	2
	Hemorrhage	3	5
	Hemosiderin pigment	1	3
	Lymphoid hyperplasia	3	1
	Reticuloendothelial hyperplasia	2	6
	Sinusoidal hystiocytosis	4	0
Colon	Parasite	13	12
Duodenum	Chronic diffuse inflammation	0	1
	Chronic inflammation	0	1
	Enterolithiasis	1	0

Organ	Lesion	Exposed	Sham
Ear	Chronic suppurative inflammation	1	0
Ear, middle	Chronic inflammation	0	1
	Chronic suppurative inflammation	1	0
	Cystic hyperplasia	0	1
Epididymus	Mucoid degeneration	1	0
	Sperm granuloma	0	1
Esophagus	Megaesophagus	1	0
Eye	Acute inflammation	3	0
	Atrophy	1	1
	Atrophy fibrosis calcification	0	1
	Calcification	1	0
	Chronic inflammation	1	1
	Chronic suppurative inflammation	5	3
	Fibrosis	0	1
	Focal granulomatous inflammation	0	1
	Hyaline degeneration	1	0
	Keratitis	7	3
Harderian G.	Chronic inflammation	0	1
	Lymphoid hyperplasia	0	1
	Porphyrin pigment	2	3
Heart	Acute inflammation	1	1
	Cardiomyopathy	29	27
	Calcification	2	2
	Cartilaginous foci	18	23
	Cartilaginous metaplasia	0	1
	Chronic inflammation	2	0
	Chronic suppurative inflammation	2	0
	Degeneration	1	0
	Fibrosis	8	8
	Hemosiderin pigment	0	1
	Hyaline degeneration	1	0
	Hypertrophy	5	6
	Thrombosis	8	11
Ileum	Chronic inflammation	0	1
Intestine	Chronic inflammation	3	0
Jejunum	Chronic inflammation	0	1

Organ	Lesion	Exposed	Sham
Kidney	Calculus	1	1
	Chronic inflammation	0	1
	Chronic progressive glomerulonephropathy	88	95
	Chronic suppurative inflammation	3	3
	Cyst	1	0
	Fibrosis and calcification	1	0
	Hydronephrosis	0	1
	Membranous glomerulonephritis	3	2
Lacrimal G.	Chronic inflammation	0	1
	Focal granulomatous inflammation	0	1
	Lymphoid hyperplasia	8	1
	Porphyrin pigment	2	0
Liver	Acute inflammation	3	1
	Acute necrotic inflammation	2	0
	Autolysis	1	0
	Bile duct ectasia	0	1
	Bile duct hyperplasia	1	2
	Biliary cyst	1	0
	Chronic focal inflammation	0	1
	Chronic inflammation	21	9
	Chronic suppurative inflammation	0	2
	Cirrhosis	0	1
	Coagulation necrosis	1	3
	Congestion	20	14
	Cyst	1	0
	Cytoplasmic vacuoles	1	0
	Fatty degeneration	0	1
	Fatty infiltration	1	0
	Fibrosis	1	0
	Focal granulomatous inflammation	3	0
	Hematopoiesis	1	0
	Hemorrhage	1	0
	Lymphocytic infiltration	0	2
	Necrosis	1	0
Lung	Acute inflammation	1	1
	Alveolar macrophage	16	17
	Atelectasis	2	2
	Calcification	0	1
	Congestion	58	58
	Edema	11	16
	Focal granulomatous inflammation	3	0
	Hemorrhage	11	10
	Hemosiderin pigment	5	2
	Interstitial pneumonia	0	1
	Lymphoid hyperplasia	75	73

Organ	Lesion	Exposed	Sham
Lymph Node	Hemorrhage	5	6
	Hemosiderin pigment	1	2
	Lymphoid hyperplasia	3	0
	Reticuloendothelial hyperplasia	1	2
	Sinusoidal hystiocytosis	3	1
Mammary	Cystic hyperplasia	0	1
	Nodular hyperplasia	0	1
Mesentery	Fat necrosis	1	0
Nasal Cavity	Calcification	5	2
	Chronic inflammation	16	8
	Chronic suppurative inflammation	7	6
	Hemorrhage	1	0
	Hematoidin pigment	1	0
	Hemosiderin pigment	10	5
Pancreas	Cyst	1	0
	Cystic ducts	0	3
	Fibrosis	1	0
	Calcification	1	0
Parathyroid	Cytoplasmic vacuoles	1	0
	Fibrosis	1	2
Parotid SG	Chronic inflammation	3	0
	Fibrosis	0	1
	Lymphoid hyperplasia	5	2
Pineal	Basophilic bodies	1	0
	Calcification	0	1
Pituitary	Calcification	0	1
	Cyst	27	16
	Focal hyperplasia	1	4
	Hemosiderin pigment	0	1
	Hyperplasia	1	1
Preputial G.	Calcification	1	0
	Chronic inflammation	15	9
	Chronic suppurative inflammation	9	9
	Cyst	2	3
	Cystic degeneration	2	0
	Cystic hyperplasia	15	16
	Hyperplasia	1	0

Organ	Lesion	Exposed	Sham
Prostate	Atrophy	3	3
	Calcification	4	3
	Calculus	1	1
	Chronic inflammation	6	7
	Chronic suppurative inflammation	4	4
	Cyst	0	3
	Proteinaceous calculus	0	1
Skeletal Mus	Calcification	1	0
	Degeneration	1	0
Skin	Adnexal gland cyst	1	0
	Cyst	1	0
	Epidermal inclusion cyst	2	1
	Focal granulomatous inflammation	1	0
Spinal Cord	Kyphosis, scoliosis	0	1
Spleen	Congestion	1	6
	Extramedullary hematopoiesis	1	0
	Hemosiderin pigment	41	43
	Hyperplasia	0	1
	Lymphoid hyperplasia	0	2
	Reticuloendothelial hyperplasia	2	2
Stomach	Calcification	2	0
	Cyst	1	1
	Hyperkeratosis	1	0
SubQ Tissue	Verminous arterial plexus	0	1
Submax SG	Chronic inflammation	0	3
	Lymphoid hyperplasia	0	1
Testes	Atrophy	15	17
	Calcification	5	4
	Chronic focal inflammation	0	1
	Chronic inflammation	0	1
	Fat necrosis	0	1
	Fibrosis	1	0
	Focal granulomatous inflammation	1	0
	Hemorrhage	1	0
	Hypoplasia	0	1
Thymus	Atrophy	1	0
	Cyst	0	1
	Hemorrhage	1	1
Thyroid	Atrophy	79	85
	C-cell hyperplasia	12	8
	Keratin cyst	0	2

Organ	Lesion	Exposed	Sham
Trachea	Chronic inflammation	1	0
	Lymphoid hyperplasia	1	0
Urethra	Calculus	1	5
	Proteinaceous calculus	0	4
	Proteinaceous plug	2	0
Urin/Bladder	Acute hemorrhagic inflammation	1	1
	Calculus	13	15
	Chronic inflammation	3	4
	Chronic suppurative inflammation	4	1
	Diffuse hyperplasia	2	0
	Hemorrhage	1	0
	Hyperplasia	1	1
	Lymphoid hyperplasia	0	1
	Nodular hyperplasia	0	1
	Rupture	0	1
Zymbal's G.	Chronic inflammation	20	20
	Lymphoid hyperplasia	17	12
	Porphyrin pigment	2	4
Total		1034	958

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